Remarks

Amendments

Claim 2 has been amended to recite that the polymerase chain reaction (PCR) reagents digested with the restriction endonuclease include a pair of primers of which "both primers of said pair of primers have" no recognition sites for the restriction endonuclease. The amendment is supported by the specification which discloses, "The primers have no recognition sites for the restriction endonuclease." Page 3, lines 9-10.

Claims 2 and 23 have been amended to indicate the product of certain of the method steps. These amendments are supported by the specification which discloses:

> The 100 ul PCR reaction cocktail contained 2.5 units of HotStarTaq DNA polymerase (QIAGEN, Inc. 25 pmol of each primer, 200 μM of each nucleotide, 4mM $MgCl_2$, and 1X PCR buffer (QIAGEN, Inc.). Restriction digestion with AluI was carried out by adding 10.0 units of enzyme to each 100 μM PCR reaction and incubating at 37°C for 1.5 hours, to allow for complete digestion of endogenous background DNA. Inactivation of restriction enzyme was achieved by heating to at 65°C for 30 min. prior to the addition of target DNA for PCR amplification.

Page 12, line 21 to page 13, line 4.

Claim 7 has been amended to recite that the primers included in the reagents for PCR "comprise" sequences as shown in SEQ ID NO: 1 and SEQ ID NO: 2 rather than "have" sequences as shown in SEQ ID NO: 1 and SEQ ID NO: 2. Claim 23 has similarly been amended to recite primers "comprising" sequences selected from the group consisting of (a) SEQ ID NO: 1 and 2; and (b) SEQ ID NO: 3 and 4 in place of primers "having" sequences selected from the group consisting of (a) and (b). These amendments are supported by the specification at page 7, lines 23-25: "More preferably the conserved regions comprises at least 18 contiguous base pairs which are at least 80% identical to PEU7 and PEU 8 (SEQ ID NO: 1 and 2), or PEU 4 and 5

(SEQ ID NO: 3 and 4)." These amendments do not narrow the scope of claims 7 and 23.

None of these amendments introduce new matter.

The Objection to Claim 2

Claim 2 has been objected to as being informal for reciting a "pair of primers have has." Claim 2 has been amended to recite that the "pair of primers have."

Applicants respectfully request withdrawal of this objection.

The Rejection of Claims 2-23 Under 35 U.S.C. § 112, Second Paragraph

Claims 2-23 have been rejected as indefinite on two separate grounds. Each will be discussed in turn.

A. Claims 2-22 have been rejected as indefinite because it is unclear whether recitation that "said pair of primers have has no recognition sites for the restriction endonuclease" indicates that one or both primers of the primer pair lack recognition sites for the restriction endonuclease. Claim 2 has been amended to recite that "both primers of said pair of primers have no recognition sites for the restriction endonuclease."

B. Claims 7 and 23 have been rejected as indefinite because it is unclear whether the primers employed in the methods comprise or consist of the recited nucleotide sequences. Claim 7 has been amended to recite that the reagents for PCR include primers that "comprise" sequences as shown in SEQ ID NO: 1 and SEQ ID NO: 2 in place of "have" these sequences. Claim 23 has been amended to recite that the reagents for PCR include a pair of primers "comprising" sequences selected from the group consisting of (a) SEQ ID NO: 1 and 2; and (b) SEQ ID NO: 3 and 4 in place of "having" sequences selected from one of (a) and (b).

Applicant respectfully requests withdrawal of these rejections.

The Rejection of Claims 2 and 4-22 Under 35 U.S.C. § 103(a)

Claims 2 and 4-22 have been rejected under 35 U.S.C. § 103(a) as unpatentable over Hoshina et al. (U.S. Patent No. 5,571,674; "Hoshina") in view of Dougherty *et al.* (*J. Virol. Methods* (1993) *41*:235-238; "Dougherty"). Applicant respectfully traverses.

Claim 2, the only independent claim of the rejected claim set, is directed to a method of performing PCR. Reagents for PCR are digested with a restriction endonuclease to form digested reagents. The reagents comprise Taq DNA polymerase, deoxynucleotide triphosphates, reaction buffer, and a pair of primers. The restriction endonuclease does not cleave the pair of primers and both primers of the pair of primers have no recognition sites for the restriction endonuclease. The restriction endonuclease is then inactivated but the Taq DNA polymerase is not inactivated to form endonuclease-inactivated digested reagents. A test sample is mixed with the endonuclease-inactivated digested reagents for PCR to form a mixture. The mixture is subjected to conditions such that any templates present in the test sample which hybridize to both primers of the pair of primers are amplified. An amplification product is detected. A detected amplification product indicates the presence of template which hybridizes to both primers in the test sample.

To reject claims as *prima facie* obvious the Patent Office must meet three criteria:

> First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

MPEP § 2143.

The Patent Office has failed to make a *prima facie* case of obviousness of claims 2 and 4-22 because the Patent Office has failed to meet the third criterion, *i.e.*, the combination of Hoshina and Dougherty fails to teach or suggest all the elements recited in claims 2 and 4-22.

Hoshina teaches a method of performing PCR. However, Hoshina does not teach or suggest digesting the reagents for PCR with a restriction endonuclease. The Office Action acknowledges that Hoshina does not teach this step. The Office Action states, "Hoshina et al. did not teach digesting PCR mixture comprising Taq polymerase, deoxynucleotides (dNTPs), reaction buffer and a pair of primers using a restriction enzyme, that do not cleave said pair of primers." Office Action at page 5, lines 5-7. Because Hoshina does not teach or suggest digesting the reagents for PCR with a restriction endonuclease, Hoshina also does not teach or suggest "mixing a test sample and the endonuclease-inactivated digested reagents to form a mixture."

Dougherty teaches a method of reverse transcription-PCR (rt-PCR). Dougherty, like Hoshina, does not teach or suggest "mixing a test sample and the endonuclease-inactivated digested reagents to form a mixture." Dougherty teaches a method of performing rt-PCR that includes steps of:

- reverse transcribing a specimen containing RNA,
- mixing the reverse transcribed specimen, *i.e.*, template, with a PCR cocktail,
- digesting the mixture of PCR cocktail and reverse-transcribed specimen with a restriction endonuclease,
- heat inactivating the restriction endonuclease,
- and amplifying the reverse-transcribed specimen.

Dougherty teaches:

> Dilutions of RNA from RV [rubella virus]-infected Vero cells (initial concentration = 10 ng/test) were reverse transcribed in 10 µl volumes and mixed with 40 µl of PCR cocktail either with or without 1.0 U of *Sma*I. The mixtures were processed and analyzed as described in the preceding paragraph. [The mixtures were processed by incubation at 30°C for 60 min to allow *Sma*I digestion, heated to 96°C for 5 min to inactivate *Sma*I, and then amplified for 30 cycles. See page 237, lines 22-25.]"

Page 238, lines 1-3. Thus, Dougherty teaches digesting a mixture of the test sample (reverse transcribed RNA from RV-infected Vero cells) with PCR reagents (PCR cocktail). Dougherty does not teach mixing endonuclease-inactivated digested reagents with a test sample as recited in independent claim 2.

Dougherty also does not suggest mixing the endonuclease-inactivated digested reagents with a test sample. In fact, Dougherty teaches mixing the test sample with the PCR reagents before restriction endonuclease digestion to eliminate all possible sources of DNA template contamination during rt-PCR. Dougherty teaches:

> Restriction endonuclease digestion was used to eliminate false-positive signals caused by polymerase chain reaction (PCR) product DNA contamination in a reverse transcribed (RT) PCR for amplifying rubella virus (RV) RNA sequences. . . Because restriction enzymes generally react only with specific double-strand sequences, contaminating DNA was rendered inactive while reverse-transcribed single strand cDNA was amplified.

Page 235, Abstract, lines 1-9. Dougherty specifically teaches digesting a mixture comprising the test sample in order to eliminate contamination. In contrast, the claimed method does not recite digestion of the test sample, but only mixes the test sample with the reagents after digestion of the reagents.

Thus, the combination of Hoshina and Dougherty fails to teach or suggest "mixing a test sample and the endonuclease-inactivated digested reagents to form a mixture" as recited in claim

2 and dependent claims 4-22. The combination of Hoshina and Dougherty fails to teach or suggest all the elements of claims 2 and 4-22 and the *prima facie* case of obviousness must fail.

Applicant respectfully requests withdrawal of this rejection.

The Rejection of Claims 3 and 23 Under 35 U.S.C. § 103(a)

Claims 3 and 23 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Hoshina in view of Dougherty and further in view of Stratagene Catalog (Stratagene Catalog, pages 301-303, 306-308, 1995; "Stratagene").

Claim 3 depends from claim 2. Claim 2, discussed above, is directed to a method of performing polymerase chain reaction that includes a step of digesting reagents for PCR with a restriction endonuclease. Claim 3 specifies that the restriction endonuclease is AluI. Claim 23 is an independent claim directed to a method of performing PCR. The method is similar to claim 2 but specifies, *inter alia*, that the restriction endonuclease is AluI. Stratagene is cited as teaching restriction endonuclease AluI and properties of AluI.

To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

Independent claim 2, from which claim 3 depends, recites a method of performing polymerase chain reaction that includes a step of "mixing a test sample and the endonuclease-inactivated digested reagents to form a mixture." As discussed above, the combination of Hoshina and Dougherty does not teach or suggest this step. Hoshina does not teach employing a restriction endonuclease in his amplification method. Thus, Hoshina does not teach or suggest mixing a test sample with endonuclease-inactivated endonuclease digested reagents to form a

mixture. Hoshina merely teaches directly mixing PCR reagents with a test sample and subjecting the mixture to amplification conditions. Hoshina teaches,

> a highly sensitive method for detecting the presence of bacteria or protozoa in a sample of a isolated DNA comprising contacting the sample with DNA oligomers which are polymerase chain reaction primers and are complementary to DNA sequences encoding universal bacterial and protozoan 16S ribosomal RNA sequences, ... , under conditions suitable for a polymerase chain reaction so as to amplify the bacterial or protozoan species-specific DNA lying between the binding sites of the DNA oligomers, and detecting the resulting species-specific bacterial or protozoan DNA sequences.

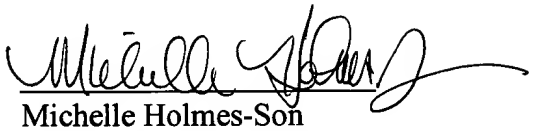
Column 7, lines 20-35.

Dougherty teaches the use of a restriction endonuclease in an amplification method. However, Dougherty teaches digesting a mixture of test sample and PCR reagents with a restriction endonuclease. Dougherty teaches, "Dilutions of RNA from RV-infected Vero cells (initial concentration = 10 ng/test) were reverse transcribed in 10 µl volumes and mixed with 40 µl of PCR cocktail either with or without 1.0 U of *Sma*I." Page 238, lines 1-3.

Stratagene also fails to teach or suggest "mixing a test sample and the endonuclease-inactivated digested reagents to form a mixture." Thus Stratagene fails to remedy the deficiency of Hoshina and Dougherty. Stratagene teaches restriction endonuclease AluI and properties of AluI. Stratagene does not teach a method of performing PCR or suggest the use of AluI in a method of performing PCR. Thus the combination of Hoshina, Dougherty, and Stratagene fails to teach or suggest a method comprising a step of "mixing a test sample and the endonuclease-inactivated digested reagents to form a mixture." As the combination of Hoshina, Dougherty, and Stratagene fails to teach or suggest this step of claim 2, it also fails to teach or suggest these steps of dependent claim 3 or independent claim 23. The *prima facie* case of obviousness must fail.

Applicant respectfully requests withdrawal of this rejection.

Respectfully submitted,

By: 

Michelle Holmes-Son
Registration No. 47,660

Date: May 9, 2005

Customer No. 22907